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Occurrence and Distribution of Actinomycetes in Marine Environs and their Antagonistic Activity against Bacteria that is Pathogenic to Shrimps

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Abstract

The mean population density of actinomycetes in water samples from eight stations off Little Andaman Island in India ranged from 0.29×10^3 CFU/ml at station 2 (Naval Area) to 0.45×10^3 CFU/ml at station 4 (Chandra Nallah). Density in sediment samples ranged from 1.21×10^3 CFU/g at station 2 (Naval area) to 3.29×10^3 CFU/g at station 6 (Buttler Bay). Forty-one strains were isolated and tested for their antagonistic activity against *Vibrio alginolyticus*, *V. harveyi*, and *V. parahaemolyticus*, bacteria that are highly pathogenic to shrimps. Over 60% of the strains (26) exhibited varying degrees of antagonistic activity. Among them, six showed good activity and were tentatively identified as *Streptomyces xantholiticus*, *S. aureofasciculus*, *S. galtieri*, *S. vastus*, *S. galbus*, and *S. rimosus*. Results suggest that actinomycetes from the marine environment can be used as bio-control agents in shrimp culture systems to control diseases caused by bacterial pathogens.

Introduction

Disease control has become a major problem in shrimp culture systems as bacterial pathogens have become more and more resistant to conventional therapeutic drugs used by the industry. Shrimp farmers are suffering heavy financial losses and there is a need to find novel bioactive compounds with therapeutic potential that can be used to combat disease. In this respect, actinomycetes, which are known to produce potent antibi-

otics, deserve to be studied. Compared to terrestrial species, actinomycetes from marine environs are important sources of novel antibiotics (Okami, 1986). Actinomycetes from marine and coastal ecosystems may provide a rich gene pool containing isolates capable of producing useful metabolites (Goodfellow and Haynes, 1984; Okami, 1986). Few attempts have been made to isolate marine actinomycetes (Balagurunathan, 1992; Balaguruna-

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than and Subramanian, 1998; Dhevendaran and Anie, 1999; Patil et al., 2001; Sahu et al., 2004, 2005ab, 2006; Sivakumar et al., 2005abc, 2006; Umamaheswary et al., 2005; Muthurayar et al., 2006). In the present investigation, water and sediment samples from eight stations off Little Andaman Island in India were screened and actinomycetes were isolated. The isolates were tested for antagonistic activity against pathogenic bacteria that cause economically damaging diseases in shrimps. Potent antagonistic strains were identified to the species level.

Materials and Methods

Collection of samples. Surface water and sediment samples were collected from eight stations off Little Andaman Island (Fig. 1). Water samples were collected in 100-ml sterile screw-capped bottles. Enough space was left in the bottles to allow thorough mixing. Precautionary measures prevented contamination through handling. Sediment samples were collected by inserting a polyvinyl corer (10-cm diameter) into the sediments. The polyvinyl corer was sterilized with alcohol before each sampling. The central portion of the top 2 cm of

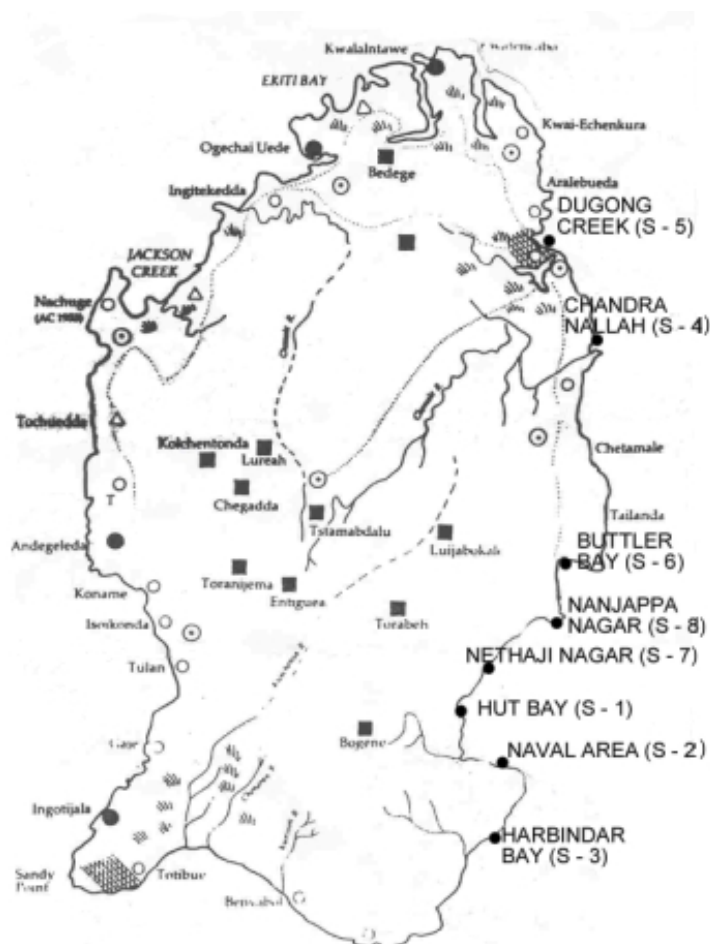


Fig. 1. Map showing eight sampling sites (S-1 through S-8) off Little Andaman Island in India.

the sediment sample was removed with a sterile spatula (Sivakumar et al., 2005a), transferred to a sterile polyethylene bag, and immediately transported to the laboratory.

Isolation of actinomycetes. The sediment samples were aseptically air-dried and, after one week, incubated at 55°C for 5 min (Waksman and Lechevalier, 1962). Both water and sediment samples were serially diluted with filtered and sterilized 50% sea water to a dilution of 10⁻². One ml of each serially diluted sample was plated in Kuster's Agar medium (pH 7.2) prepared in 50% sea-water to enhance isolation of the actinomycetes (Sivakumar et al., 2005a). Cycloheximide (100 mg/l) and nalidixic acid (20 mg/l) were added to the medium to prevent fungal and bacterial contamination (Kathiresan et al., 2005). The petri plates were incubated at 28±2°C and colonies were observed from day 5 onwards for one month (Sivakumar et al., 2005a). Strains of actinomycetes were picked out and purified by repeated streaking on a medium of yeast extract-malt extract agar (ISP 2). Pure actinomycetes cultures were transferred to ISP-2 slants and preserved at 4±2°C.

Antagonistic activity against shrimp bacterial pathogens. The antagonistic activity of the isolated actinomycetes was studied against *Vibrio alginolyticus*, *V. harveyi*, and *V. parahaemolyticus* from the Indian Institute of Microbial Technology in Chandigarh. Antagonistic activity was tested using the cross streak method (Waksman and Lechevalier, 1962). A single streak of actinomycete was made on the surface of modified nutrient agar (Sivakumar et al., 2005a) and incubated at room temperature (28±2°C). After observing good ribbon-like growth of the actinomycete on the petriplates, the pathogen was streaked at right angles to the original streak of actinomycete and incubated at 28±2°C. The inhibition zone was measured after 24 and 48 h. Uninoculated control plates were maintained to assess the normal growth of the bacteria.

Taxonomic investigation. The genus of strains with good antagonistic activity against the pathogens was identified using cell wall

composition analysis and micromorphological studies (Lechevalier and Lechevalier, 1970). The species was identified based on methods described by Shirling and Gottlieb (1966), the key of Nonomura (1974), and Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

Results

Population density. The population density of actinomycetes in water samples ranged from 0.29 x 10³ CFU/ml at station 2 to 0.45 x 10³ CFU/ml at station 4 (Fig. 2). Sediment samples ranged from 1.21 x 10³ CFU/g at station 2 to 3.29 x 10³ CFU/g at station 6.

Antagonistic activity. Twenty-six (61%) of the 41 isolated strains showed antibacterial activity against shrimp pathogens. Of these, six strains showed good activity and were selected for identification. All six showed prominent activity against *V. harveyi* and strain MKS-24 was very active against all three pathogens (Table 1).

Taxonomic identification. All six strains possessed LL-diaminopimelic acid (DAP) and glycine in the cell wall and tested negative for meso-DAP, indicating that the cell wall was of chemotype-1 that has no characteristic sugar pattern (Lechevalier and Lechevalier, 1970). Representatives of chemotype-1 include *Streptomyces*, *Streptovorticillium*, *Chainia*, *Actinopycnidium*, *Actinosporangium*, *Elytrosporangium*, *Microellobosporia*, *Sporichthya*, and *Intrasporangium* (Lechevalier and Lechevalier, 1970). Micromorphological observations of the strains revealed that all belong to the genus *Streptomyces*.

Morphological, micromorphological, physiological, and biochemical characteristics of the six strains were compared with those of *Streptomyces* in the key of Nonomura (1974) and species described in the Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). In MKS-09, all characters except the non-utilization of fructose (Table 2), were identical to those of *S. xantholiticus*. Therefore, MKS-09 was tentatively identified as *S. xantholiticus*. In MKS-13, utilization of the carbon compounds, mannitol and rhamnose, slightly differed but all other characters

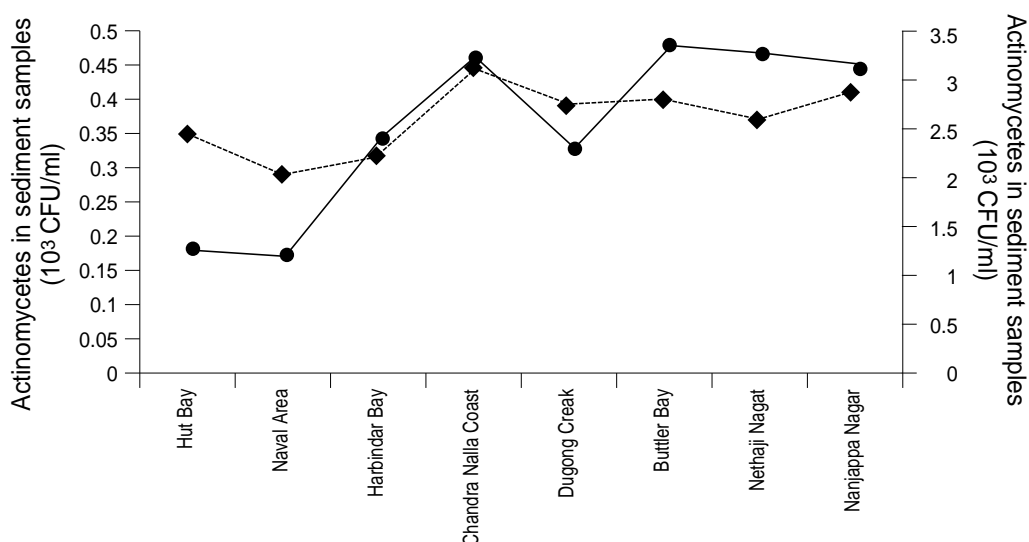


Fig. 2. Population density of actinomycetes in water and sediment samples from the east coast of Little Andaman Island, India.

Table 1. Antagonistic activity (inhibition zone in mm) of six strains of actinomycetes against shrimp bacterial pathogens.

| Actinomycete strain | Shrimp bacterial pathogen | | |
|---------------------|-----------------------------|-----------------------|--------------------------------|
| | <i>Vibrio alginolyticus</i> | <i>Vibrio harveyi</i> | <i>Vibrio parahaemolyticus</i> |
| MKS-09 | 11 | 16 | - |
| MKS-13 | - | 13 | 7 |
| MKS-17 | 5 | 7 | 15 |
| MKS-24 | 13 | 12 | 12 |
| MKS-35 | 9 | 11 | 14 |
| MKS-39 | 15 | 14 | 8 |

resembled *S. aureofasciulus*, therefore MKS-13 was tentatively identified as *S. aureofasciulus*. MKS-17 was similar to *S. gallieri*, except for utilization of sucrose and non-utilization of fructose, and therefore tentatively identified as *S. gallieri*. Except for the production of soluble pigment, all properties of MKS-24 were identical to those of *S. vastus*, therefore it was tentatively identified as *S. vastus*. MKS-35 slightly varied from *S. galbus* in utilization of xylose

and rhamnose, and was therefore tentatively identified as *S. galbus*. MKS-39 differed from *S. rimosus* only in utilization rhamnose and sucrose and was tentatively identified as *S. rimosus*.

Discussion

Population density. Actinomycete populations in estuarine and marine sediments vary in density between regions and even between

Table 2. Morphological and micromorphological characteristics and utilization of carbon compounds by six strains of antagonistic actinomycetes.

| Strain | Species to which assigned | Aerial mass color | Melanoid pigment | Reverse side pigment | Soluble pigment | Spore chain morphology | Spore surface | Utilization of carbon compounds | | | | | | | | | |
|--------|-------------------------------------|-------------------|------------------|----------------------|-----------------|------------------------|---------------|---------------------------------|---|---|---|---|---|---|----|---|---|
| | | | | | | | | A | X | I | M | F | R | S | Ra | | |
| MKS-09 | <i>Streptomyces xantholiticus</i> | Gray | - | + | - | Spiral | Smooth | - | + | ± | - | - | - | - | - | - | - |
| MKS-13 | <i>Streptomyces aureocirculatus</i> | White | + | + | + | Rectiflexible | Smooth | + | + | - | + | - | + | ± | + | | |
| MKS-17 | <i>Streptomyces galteri</i> | White | - | - | - | Rectiflexible | Smooth | - | - | - | - | - | - | - | + | - | - |
| MKS-24 | <i>Streptomyces vastus</i> | Gray | - | + | + | Rectiflexible | Smooth | + | ± | + | + | + | + | + | + | + | + |
| MKS-35 | <i>Streptomyces galbus</i> | Gray | + | + | + | Spiral | Smooth | ± | + | + | + | - | + | + | ± | | |
| MKS-39 | <i>Streptomyces rimosus</i> | Gray | - | + | + | Spiral | Smooth | + | ± | + | + | + | + | + | + | + | + |

+ = present, - = absent, ± = doubtful

A = arabinose, X = xylose, I = inositol, M = manitol, F = fructose, R = rhamnose, S = sucrose, Ra = raffinose

sites within an ecosystem. In the present study, the highest density in sediment samples was 3.29×10^3 CFU/g. In similar studies, actinomycete densities in sediment samples from marine ecosystems reached $0-15 \times 10^4$ CFU/g (Cochin, India; Kala and Chandrika, 1995), $1-4 \times 10^4$ CFU/g (Pichavaram mangrove, Tamilnadu, India; Sivakumar et al., 2005b), and up to the highest record available, 40×10^4 CFU/g (New Mexico, USA; Weyland and Helmke, 1988).

The density was higher in sediment samples than in water samples at all stations, similar to findings in the Vellar estuary on the Parangipettai coast (Sahu et al., 2005a). This agrees with observations of Goodfellow and Williams (1983) who found that the population density of actinomycetes is lower in marine sediments than in terrestrial soils. It seems that large amounts of actinomycetes are washed from the land into the sea and connected areas where only some remain viable.

Antagonistic activity. Among the 41 isolated actinomycetes, 26 strains (61%) showed antibacterial activity against shrimp pathogens. Earlier reports on marine actinomycetes also revealed prominent antagonistic activity. Sivakumar et al. (2005a) isolated 91 strains of actinomycetes from a mangrove environment, 41.67% of which showed prominent antagonistic activity. The reason for the occurrence of antagonistic actinomycetes may be that continuous fluctuations of physico-chemical parameters in the coastal environment enhance production of antagonistic substances in organisms, enabling them to survive (Sahu et al., 2006).

So far, few active compounds isolated from marine actinomycetes are known to act as potential antibiotics with broad spectral activity (Balagurunathan, 1992). Six strains of *Streptomyces* isolated in the present study showed considerable anti-bacterial activity against shrimp pathogens. These strains, especially MKS-24 which was very active against all three pathogens, should be further investigated to characterize its active compounds and as a source of potent antibiotics for combating diseases in cultured shellfish.

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